Remarks

The invention

The invention features a method for recombinase mediated expression cassette exchange (RMCE) for substituting a positive-negative selectable marker by an incoming DNA, using FLP-recombinase.

The Office Action

Claims 1 - 6, 10 and 11 are pending in this application. Claims 1 - 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Schlake and Bode (Biochemistry 33: 12746-12751, 1994).

Further, claims 1, 10 and 11 are rejected under 35 U.S.C. § 103(a) as the being unpatentable over Schlake and Bode in view of Jung et al. (Science 259:984-987, 1993) as well as Schlake and Bode in view of Ludwig et al. (Transgenic Research 5:385-395, 1996).

Claims 1 - 6, 10 and 11 are further rejected under 35 U.S.C. § 112, 1st paragraph, because the specification does not reasonably provide enablement for the practice in animals other than mice.

Additionally, claims 1 - 6, 10 and 11 stand rejected under 35 U.S.C. § 112, 2nd paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Each of these rejections are addressed below.

Support for the amendments

The new claims find support throughout the specification, in particular the following amendments of claim 1 are disclosed:

(i) Deletion of the term "repetitive"

The paragraph bridging pages 1 and 2 of the application as filed discloses both alternatives, which are mentioned by the Examiner with respect to the meaning of "repetitive". This paragraph discloses "repetitive modification (...) in a known locus ...", which means that the cassette is capable being replaced several times. Further, the same paragraph discloses "... but also randomly integrated constructs...", which means that the exchange of the cassette can happen several times. Therefore, both possibilities are disclosed, which means that "repetitive" is not a limiting feature of independent claim 1, so that deletion to clarify the meaning of the preamble is not an enlargement of the scope of protection but is fully covered by the disclosure in the specification.

(ii) Insertion of "recombinase mediated" expression cassette exchange (RMCE)

The addition of the full term of RMCE is clearly defined in the application on page 4, 3rd paragraph.

(iii) Insertion of the intended use of RMCE

The explanation of the function of RMCE, namely "for substituting a positive-negative selectable marker by an incoming DNA" is explicitly mentioned on the description on page 4, last paragraph.

(iv) Deletion of the term "tagging"

It is clear from steps (a) in combination with (c) of claim 1 that the two FLP-recombinase recognition target sites are responsible for the exchange of the two cassettes. This exchange is mediated by FLP-recombinase. As the term "for tagging" does not add any further information or imitation, it was deleted.

(v) Substitution of embryonic stem (ES) cells for regenerative cells

Claim 5 was canceled and the teaching of claim 5 was incorporated into claim 1, so that only ES cells and parts thereof can be used.

(vi) Exchange "until" for "while" in step (d)

This amendment is fully supported by page 8, last paragraph.

Amendments of claims 6 and 11 only contain minor amendments, while claim 10 has been amended in order to incorporate method steps.

The abstract of the specification has been shortened so that it now contains less than 150 words, and legal phraseology of patent claims has been omitted.

All amendments are fully supported by the application as regionally filed, all amendments are allowable as no new subject matter is added by these amendments.

Rejection under 35 U.S.C. § 112, 1st paragraph

Claims 1 - 6, 10 and 11 are rejected under 35 U.S.C. § 112, 1st paragraph. The Examiner particularly objected the term in claim 1(c) that cells used in the claimed method "can regenerate to complete organism". Therefore, claim 1 has been amended so that it now only contain embryonic stem cells, which is fully enabled by the specification. A person skilled in the art will know from the teaching of the specification and his general knowledge

about embryonic stem cells how to obtain embryonic stem cells of various animals, like mouse, sheep, goats, etc.

Further, the Examiner stated that at the moment ES cells are only available for the mouse as it is reviewed in Seamark (Reprod. Fertil. Dev., 6: 653 - 657, 1994) and Moreadith and Radford (J. Mol. Med. 75: 208 - 216, 1996). This estimation is respectfully traversed. The Examiner's attention is drawn to the fact that this application claims a foreign priority of 1998 and the cited documents were already published four and two years, respectively, before the priority date. The person skilled in the art is completely aware that embryonic stem cells at the priority date were not only available from mouse but also from other animals.

The § 112, 1st paragraph, rejection in this case should be withdrawn.

Rejection under 35 U.S.C. § 112, 2nd paragraph

Claim 1 was objected due to numerous unclear expressions, which have all been deleted and amended, so that this objection is overcome by amended claim 1. Further, claim 10 has also been amended in order to now recite method steps on how to obtain the animal.

Therefore, the § 112, 2iid paragraph, rejection should be withdrawn.

Rejection under 35 U.S.C. § 102(b)

Claims 1 - 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Schlake and Bode (Biochemistry 33: 12746 - 12751, 1994). Specifically, the Office points to the teaching of Schlake and Bode and states that this document teaches a method of RMCE as set forth in the instant specification, in particular, the Office points out that Schlake and Bode use CV- 1 and BHK cells, but Schlake and Bode teach that one can use the technique in other cell types including mouse ES cell lines. Applicants respectfully traverse this objection.

First of all, the relevant passage of Schlake and Bode, page 12746, left column, 1st paragraph, read as follows:

> For higher eukaryotes, homologous recombination is an essential event participating in processes like DNA repair and chromatid exchange during mitosis and meiosis. Recombination depends on two highly homologous, extended sequences and several auxiliary proteins, only part of which has been identified. Strand exchange can occur at any point between the regions of homology, although particular sequences may influence efficiency. These processes can be exploited for a targeted integration of transgenes into the genome of certain cell types like embryonic stem cells. On the other hand, cultured cell lines relevant for genetic engineering purposes have lost the potential to perform homologous recombination at the efficiency that would be required to incorporate it into routine procedures (S. Karreman, GBF, unpublished). We chose BHK, which is one of the two most frequently used lines in biotechnology and has a long track record for the safe production of vaccines.

From the full citation of the relevant paragraph, it is clear for a person skilled in the art that Schlake and Bode did explicitly decide to not use embryonic stem cells due to their know drawbacks. They wanted to use an established cell line, namely BHK and CV-1 cell lines because cell lines have lost the potential for performing homologous recombination. Throughout the entire document, Schlake and Bode do not use, discuss or mention the potential use of embryonic stem cells (see in particular the section "discussion" on pages 12750-12751). Therefore, Schlake and Bode do not teach the feature that the method for recombinase mediated expression cassette exchange ("RMCE") has to be performed in embryonic stem cells, as is claimed by independent claim 1.

Further, Schlake and Bode do by no means teach the features mentioned in step (d) of claim 1, namely to maintain the conditions for positive selection during cultivation of the cells obtained in step (b) until exchanging the first DNA expression cassette against the incoming second DNA expression cassette. The relevant passages in Schlake and Bode are on page 12746, right column, 2nd paragraph, where it is said that "the vector is suited for positive (hygromycin) and negative (gancyclovir) selection" and page 12747, right column, 3rd

paragraph, where it is stated that "BHK cells containing a single copy of F_5HygTKF (...) were cultured continuously for 4 weeks (...) before they were transfected with 1 μg of F_5NeoF and 2 μg of p0G44. G418-resistant clones isolated after two more weeks were characterized using PCR primers...."

From these passages, it is clear for the person skilled in the art that Schlake and Bode does not teach to maintain positive selection conditions with hygromycin until the exchange of the first DNA expression cassette against the second incoming DNA expression cassette is complete.

For anticipation under 35 U.S.C. § 102, the reference must teach every aspect of the claimed invention either explicitly or impliedly. Any feature not directly taught must be inherently present (see MPEP § 706.02). As Applicants have convincingly demonstrated that the reference Schlake and Bode does not teach every aspect of the claimed invention, the Examiner is respectfully requested to withdraw the anticipation rejection.

Rejection under 35 U.S.C. § 103

Claims 1, 10 and 11 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Schlake and Bode in view of either Jung et al. or Ludwig et al. The Office points to the fact that Schlake and Bode mention mouse ES cell lines, but they do not teach to use the method to create cells which are capable of regenerating an animal. However, Jung et al. teaches a method using an ES cell modified by FLP recombinase to generate a transgenetic animal. Therefore, the Office believes that it would have been *prima facie* obvious for a person skilled in the art at the time the invention was made to use the methods of Schlake and Bode to modify the genome of an ES cell using FLP recombinase to create a transgenic animal as described by Jung et al.

Further, the Office believes that Ludwig et al. is equally suitable to be combined with the disclosure of Schlake and Bode, because Ludwig et al. teaches a method to modify

the genome of a fertilized one cell egg using FLP recombinase. Therefore, it would have been *prima facie* obvious for a person skilled in the art at the time the invention was made to use the methods of Schlake and Bode to modify the genome of fertilized egg using FLP recombinase to create a transgenic animal as described by Ludwig et al. Applicants respectfully traverse this rejection.

To make out a *prima facie* case of obviousness, the Patent Office must show that "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person skilled in the art to which said subject matter pertains." 35 U.S.C. § 103(a) (emphasis added). The framework for making such a determination was set out by the United States Supreme Court in *Graham v. Deere Co.*, 383 U.S. 1, 17-18 (1966):

> Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined.

Obviousness cannot be established by combining the teaching of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. *ASC Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 U.S.P.Q. 929, 933 (Fed. Cir. 1984). This suggestion or motivation may be derived from the prior art reference itself, from the knowledge of one of ordinary skill in the art, or from the nature of the problem to be solved. The basis for a prior art combination, however, must come from a source other than the inventor's disclosure. The Court of Appeals for the Federal Circuit has repeatedly emphasized that hindsight analysis is an inappropriate means for piecing together the elements of an invention from unrelated references. For example, in *In re Fritch*, 972 F.2d 1260, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992), the Federal Circuit stated:

> It is impermissible to use the claimed invention as an instruction manual or 'template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious.

And in *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed. Cir. 1985), the court stated:

> It is error to reconstruct the patentee's claimed invention from the prior art by using the patentee's claim as a "blueprint". When prior art references require selective combination to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight obtained from the invention itself.

Applying these standards to the present case, it is clear that the references cited in the latest Action do not support a *prima facie* case of obviousness.

As discussed above, Applicants' claimed invention is a method for recombinase mediated expression cassette exchange (RMCE) using a first DNA expression cassette which is exchanged against an incoming second DNA expression cassette by simultaneously positive-negative selection and using FLP-recombinase. The entire method is conducted in embryonic stem cells or part of these cells.

Schlake and Bode, as already discussed above, clearly states to not use embryonic stem cells but rather suggests to use established cell lines like BHK or CV-1 cells due to their known advantages. Therefore, a person skilled in the art would never have started with the document of Schlake and Bode and combine it with either Jung et al. or Ludwig et al. Additionally, both references, Jung et al. and Ludwig et al., use FLP-recombinase for excision of a sequence between two identical, equally orientated FRT sites. In contrast to this, the invention and in particular independent claim 1 in step (a) and step (c) clearly disclose to use one wild type FLP-recombinase recognition target site and a second modified heterospecific FRT site, which is clearly not suggested by Jung et al. or Ludwig et al. The methods taught by Jung et al. or Ludwig et al. are a three step procedure comprising the steps of

a) excision (or inversion) between two identical FRT-sites, which is a kinetically and thermodynamically favored monomolecular reaction, which, by nature, is highly efficient;

(b) integration, i.e. recombination between a single genomically anchored FRT site and an alike site on a targeting vector. This bimolecular reaction is highly inefficient. It integrates all vector sequences and here, integration automatically leads to the scenario described under (a), i.e. a situation, which is spontaneously reversed unless the recombinase can be inactivated at the moment this step has occurred; and

(c) the exchange reaction (RMCE), which was first demonstrated by Schlake and Bode, 1994.

What was entirely surprising and was demonstrated by Southern Plot hybridization, that RMCE reaction can proceed with up to 40% efficiency since it can be enriched by negative selection. This was totally unexpected in view of the previous work of Schlake and Bode as it were expected that any crossinteraction between the heterospecific sites (e.g. F3 and F, respectively), would lead to an excision of the cassette according to the principal (a) as mentioned above. Negative selection would in this case enrich for excision events rather than desired exchange (RMCE). It is noted in this respect that in the PCR analysis of transient recombination events (Schlake and Bode, Biochemistry 33: 12746-12751, 1994; Fig. 3) F3-F crossinteraction is visible.

A person skilled in the art may thus not have been able to reach the claimed invention by combining the teaching of Schlake and Bode with either Jung et al. or Ludwig et al.

The § 103(a) rejection in this case should be withdrawn.

Last paragraph of the Office Action

The Examiner stated that the prior art reference of Wahl et al. (U.S. patent 5,654,182) is considered pertinent to Applicants' disclosure but is not applied against the claims.

Applicants have no further comments on this reference, except to say that it is not believed relevant to the present invention as claimed.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such Action is respectfully requested.

A check in the amount of $465.00 is enclosed to cover the Petition fee. Please charge any additional fees or credit any overpayments as a result of the filing of this paper to our Deposit Account No. 02-3978 -- a duplicate of this paper is enclosed for that purpose.

Respectfully submitted,

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